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mation and the resultant characteristic and perhaps objectionable odor found in thiamin and thiamin containing vitamin preparations.

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A New Method of Pyrazine Synthesis for Flavor Use

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Synthesis of alkylpyrazines from α-diketones and α-diamines through the corresponding 2,3-dihydropyrazines was studied. Dehydrogenation of dihydropyrazines in an ethanol–KOH solution with the addition of metal oxides (MnO_2, CuO) gave nearly 80% yields of pyrazines. The pyrazines obtained from this study were over 99% pure and did not contain any unreacted dihydropyrazines or by-products which cause changes in either the color or odor of the pyrazines. A kinetic study of trimethyl- and 2,3-dimethylpyrazine formation from corresponding pyrazines was also conducted.

Pyrazine compounds have been well characterized as the compounds which directly contribute to roasted or smoky flavors. There have been many reports concerning these flavor compounds (Bondarovich et al., 1967; Rizzi, 1967; Watanabe and Sato, 1971; Buttery et al., 1971). Formation mechanisms of pyrazines in foods have been investigated by several researchers (Koehler and Odell, 1970; Rizzi, 1972; Wang and Odell, 1973; Shibamoto and Bernhard, 1976).

The most common method for deriving pyrazines from 2,3-dihydropyrazines, which are obtained from the condensation reaction of α-dicarbonyl compounds and α-diamines, is to heat the 2,3-dihydropyrazines in an alcoholic solution under basic conditions in order to dehydrogenate them. For example, Flament and Stoll (1967) obtained 3-alkyl-2-methylpyrazine from the reaction of 2,3-dioxoalkanes and ethylenediamine through 2,3-dihydropyrazines. Their method gives a 55–100% yield. This experimental procedure is, however, somewhat difficult and requires a high temperature, which may cause the production of unpleasant odor. Nakatani and Yanatori (1973) obtained dialkylpyrazines from corresponding dihydropyrazines in 40–75% yields using KOH or NaOH in ethylene glycol. It is, however, difficult to separate pyrazines from ethylene glycol and this method produces a certain amount of by-products which are not suitable for flavor use. Ishiguro and Matsumura (1958) obtained 2,3-dimethylpyrazine from the reaction of diacetyl and ethylenediamine through 2,3-dimethyl-5,6-dihydropyrazine. They treated 2,3-dimethyl-5,6-dihydropyrazine with potassium hydroxide in ethanol at high temperature to dehydrogenate it. Cornforth (1958) reported the syntheses of symmetric alkylpyrazines from the self-

condensation reaction of α-amino ketones through dihydropyrazines. Dihydropyrazines were either dehydrogenated by an oxidizing agent (H_2O_2, $HgCl_2$, etc.) or oxidized under aerobic conditions. Tutin (1910) obtained a mixture of equal amounts of 2,5- and 2,6-diphenylpyrazine through corresponding dihydropyrazines from the reaction of ω-chloroacetophenone with alcoholic ammonia. These methods either produced low yields or the pyrazines formed could not be easily separated from the unreacted dihydropyrazines. It is, therefore, very difficult to obtain high-purity pyrazines for flavor usage via these methods.

We used a modification of Ishiguro's method in order to obtain high yields of alkylpyrazines of very high purity.

EXPERIMENTAL SECTION

Materials. Diacetyl (Naarden), ethylenediamine, propylenediamine (Nakarai Chemicals, Ltd.), metal oxides, and metal salts (Wako Pure Chemical Industries) were obtained commercially. Acetylpropionyl was synthesized using the method reported by Semon and Damerell (1930). Dihydropyrazines were synthesized by the procedures described by Ishiguro and Matsumura (1958).

Synthesis of Pyrazines from Corresponding Dihydropyrazines. Five grams of corresponding dihydropyrazine was dissolved in 63 mL of ethanol for each experiment. *n*-Nonanol (2 g) was added to each of these solutions as the internal standard for gas chromatographic quantitation. The above solutions were refluxed with the materials and under the conditions as described in Table I. After refluxing, each reaction solution was filtered, and 5 mL of each filtrate was mixed with 20 mL of benzene and 5 mL of saturated brine water and shaken vigorously. The quantitative analysis of the pyrazine in the benzene layer of each of the above solutions was conducted by gas chromatography.

Preparation of Standard Trimethylpyrazine from Trimethyl-5,6,-dihydropyrazine for Flavor Use.
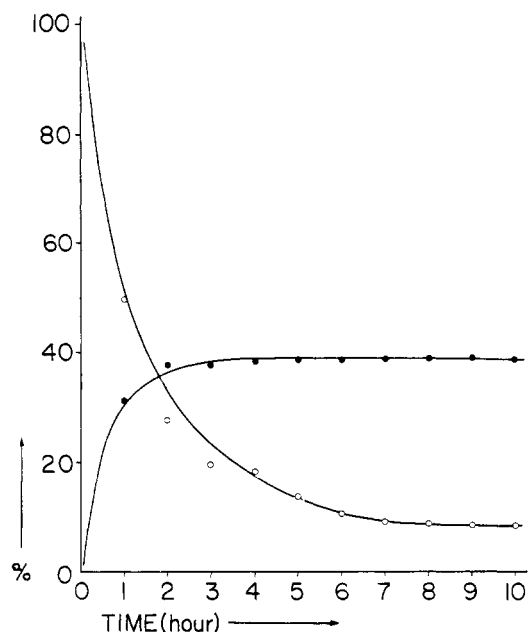
Figure 1. Formation of trimethylpyrazine without metal oxides: (●) pyrazine produced, (○) unreacted dihydropyrazine.

Figure 3. Formation of trimethylpyrazine in the presence of MnO₂: (●) pyrazine produced, (○) unreacted dihydropyrazine.

Figure 2. Formation of trimethylpyrazine in the presence of CuO: (●) pyrazine produced, (○) unreacted dihydropyrazine.

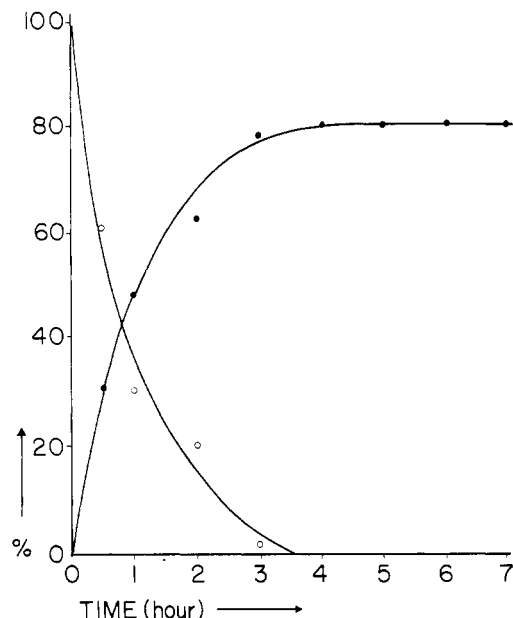**Figure 4.** Formation of 2,3-dimethylpyrazine without metal oxides: (●) pyrazine produced, (○) unreacted dihydropyrazine.

Trimethyl-5,6-dihydropyrazine (100 g) was dissolved in 1260 mL of ethanol. KOH (50 g) and MnO_2 (210 g) were added to this ethanol solution. The above solution was refluxed for 7 h and stirred with a magnetic stirrer. The reaction mixture was filtered and the filtrate concentrated to a 250-mL volume using a rotary flash evaporator. Saturated brine water (250 mL) was added to the above solution, and the solution was extracted with two 500-mL portions of benzene and the extract was dried over anhydrous magnesium sulfate for 12 h. Trimethylpyrazine was isolated by fractional distillation under reduced pressure: boiling point, 90–92 °C at 50 mmHg. The yield of trimethylpyrazine was 82.3% (81 g). 2,3-Dimethylpyrazine was isolated in the same way as described above: yield, 77.4%; boiling point, 75–78 °C at 50 mmHg.

Kinetics. In order to investigate the kinetics of pyrazine formation from dihydropyrazine, the following experiments were conducted. Dihydropyrazines and metal oxides in
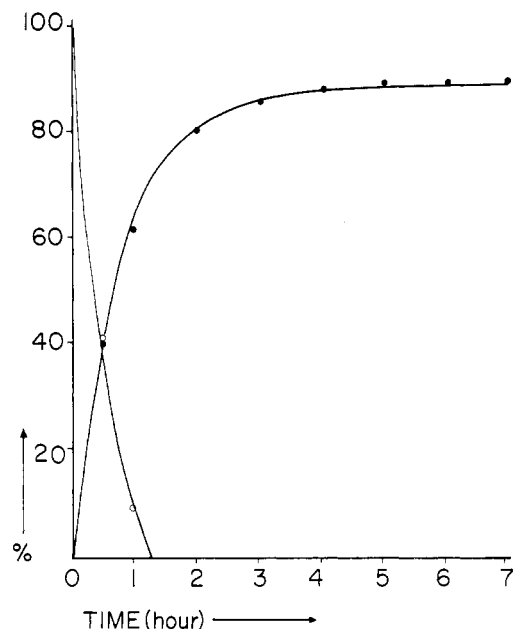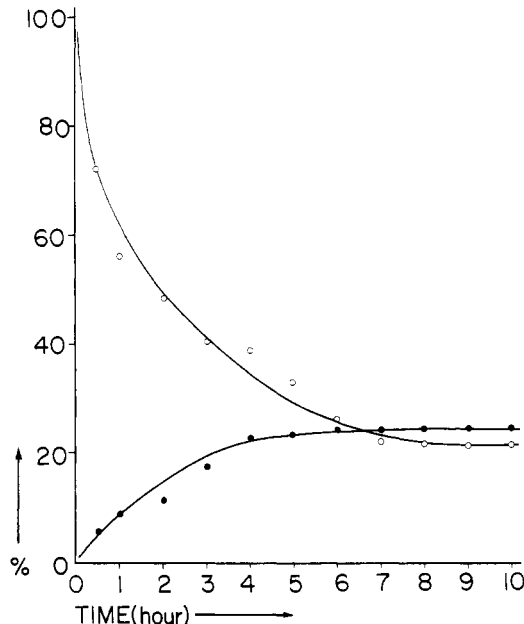
1:3 molar ratios were refluxed in an ethanol–KOH solution. Trimethyl-5,6-dihydropyrazine and 2,3-dimethyl-5,6-dihydropyrazine were each refluxed with CuO and MnO_2. The amounts of pyrazine formed and amounts of dihydropyrazine remaining unreacted were measured every hour for 7 h by the method described above (experiment no. 1 and 21 were run for 10 h). The results are shown in Figures 1–6.

RESULTS AND DISCUSSION

The results obtained from this study are shown in Table I.

Addition of Metal Oxides. It is quite obvious that the yield of pyrazine increased with the addition of metal oxides. In particular, the addition of MnO_2 or CuO doubled the yield (experiment no. 3–9). On the other hand, TiO_2 (experiment no. 14) and BaO_2 (experiment no. 15) did not influence the yield of pyrazine.

Table I. Reaction Conditions and Experimental Results

exp. no.	dihydropyrazine used	added material	amount of added material[a]	reaction time, h	amount of KOH[b]	presence of unreacted dihydropyrazine	yield, %[c]
1	trimethyl-	blank		1-10	1.10	refer to Fig. 1	refer to Fig. 1
2		CuO	0.3	5	1.10	–[d]	35.7
3		CuO	1.0	5	1.10	–	58.6
4		CuO	2.0	5	1.10	–	57.8
5		CuO	3.0	1-7	1.10	refer to Fig. 2	refer to Fig. 2
6		CuO	3.0	5	0.44	–	80.1
7		CuO	3.0	5	blank	+[e]	none
8		MnO$_2$	1.5	5	1.10	–	74.2
9		MnO$_2$	3.0	1-7	1.10	refer to Fig. 3	refer to Fig. 3
10		PbO$_2$	1.0	5	1.10	–	55.5
11		PbO$_2$	3.0	5	1.10	–	61.0
12		Pb$_3$O$_4$	1.5	5	1.10	–	62.7
13		Pb$_3$O$_4$	3.0	5	1.10	–	70.8
14		TiO$_2$	3.0	5	1.10	+	39.9
15		BaO$_2$	3.0	5	1.10	+	37.3
16		Cu(OAc)$_2$·H$_2$O	3.0	5	1.10	–	none
17		CuSO$_4$·5H$_2$O	3.0	5	1.10	–	none
18		H$_2$O$_2$	1.0 (10%)	5	1.10	+	52.4
19		H$_2$O$_2$	3.0 (10%)	5	1.10	+	48.7
20		H$_2$O$_2$	3.0 (30%)	5	1.10	+	58.9
21	2,3-dimethyl-	blank		1-10	1.0	refer to Fig. 4	refer to Fig. 4
22		CuO	3.0	1-7	1.0	refer to Fig. 5	refer to Fig. 5
23		MnO$_2$	3.0	1-7	1.0	refer to Fig. 6	refer to Fig. 6

[a] Molar ratio of the added material relative to dihydropyrazine used. [b] Molar ratio of KOH relative to dihydropyrazine. [c] (The amount of pyrazine produced)/(the amount of dihydropyrazine used) × 100. [d] Not present. [e] Present.
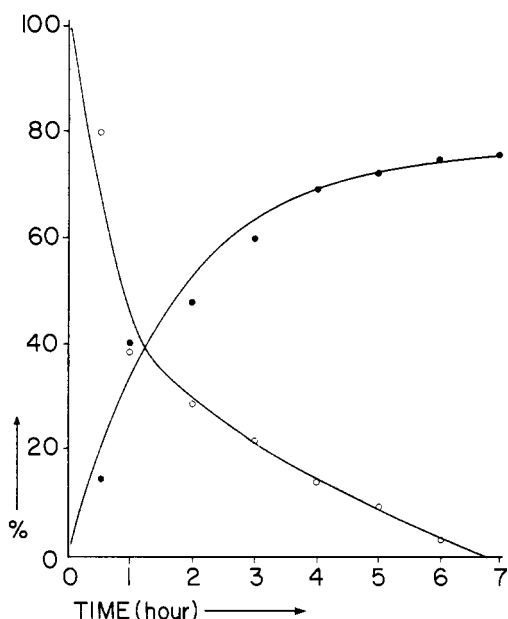

Figure 5. Formation of 2,3-dimethylpyrazine in the presence of CuO: (●) pyrazine produced, (○) unreacted dihydropyrazine.

Figure 6. Formation of 2,3-dimethylpyrazine in the presence of MnO$_2$: (●) pyrazine produced, (○) unreacted dihydropyrazine.

Addition of Metal Salts. The results indicate that excess metal oxides were necessary in order to obtain better yields. If the amount of a metal oxide used was less than 1 in molar ratio relative to dihydropyrazine, then the positive effect on yield was not observed (experiment no. 2). These results also indicate that the metal oxides act as oxidizing agents rather than catalysts. Oxidation occurs on the surface of the oxidant after the material is adsorbed on the surface of the oxidant. The oxidant, therefore, is required to be present in quantities greater than 1 in molar ratio relative to the dihydropyrazine used. Pyrazines did not form when Cu(OAc)$_2$·H$_2$O or CuSO$_4$·5H$_2$O was added (experiment no. 16 and 17). In these cases polymers were formed, and the reactant dihydropyrazine was not recovered. This is reasonable because when a metal salt is added to an ethanol–KOH solution, it reacts with the KOH
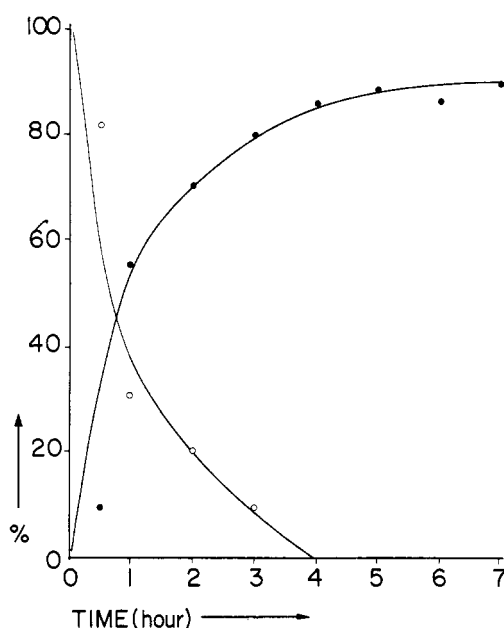
and the pH of the solution decreases. It is essential to keep the solution basic when a metal salt is added because the stability of a dihydropyrazine is dependent on the basicity of the solution. It is necessary, therefore, to select a salt which is stable in basic solution.

Addition of Hydrogen Peroxide. Hydrogen peroxide does not change the pH of the solution and has a slight effect on pyrazine yield (experiment no. 18–20), but less than that of the metal oxides. Thirty percent hydrogen peroxide (experiment no. 20) also gave a higher yield than did 10% hydrogen peroxide.

Kinetics. The formation of trimethylpyrazine in the absence of metal oxides leveled off after 3 to 4 h reaction time, and the yield reached only 40% (Figure 1). Unreacted trimethyl-5,6-dihydropyrazine remained at about 10%. The oil recovered by distillation from the blank run

became brown and possessed an unpleasant, amine odor after several days due to the polymerization or decomposition of unreacted trimethyl-5,6-dihydropyrazine. In the case of CuO addition (Figure 2), the formation of the pyrazine leveled off after 3.5 h of refluxing. Maximum yield peaked at 80% and trimethyl-5,6-dihydropyrazine disappeared completely. Also, the yield of pyrazine leveled off at 90% after 2 h and trimethyl-5,6-dihydropyrazine disappeared completely when MnO_2 was added (Figure 3). The formation of 2,3-dimethylpyrazine from 2,3-dimethyl-5,6-dihydropyrazine behaved in exactly the same manner as that of the formation of trimethylpyrazine in solutions containing no metal oxides, CuO, and MnO_2 (Figure 4, 5, and 6, respectively). It should also be pointed out that the rates of pyrazine formation in experiment no. 1, 5, 9, 21, 22, and 23 satisfy the equation of first-order reaction kinetics, which agrees with the results reported previously (Shibamoto, 1975).

CONCLUSION

The final reaction solution prepared by this method does not contain other pyrazines or dihydropyrazines. Therefore, pyrazines obtained from distillation of these solutions are over 99% pure and do not change their color or odor over long periods of storage. The best yield of a pyrazine was obtained from the oxidation of the corresponding dihydropyrazine in ethanol–KOH solution with a 3 molar ratio of CuO or MnO_2 to the dihydropyrazine. Pyrazines obtained by this method are useful as flavor ingredients because of their high purity and stability.

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Formation of Heterocyclic Compounds from the Reaction of Cysteamine and D-Glucose, Acetaldehyde, or Glyoxal

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The volatile compounds produced from the reaction of cysteamine and D-glucose, acetaldehyde, or glyoxal were extracted with methylene chloride using a liquid–liquid continuous extractor. Gas chromatographic and mass spectrometric methods were used to identify 24 compounds. The compounds identified were mainly heterocyclic compounds including pyrazines, thiazoles, thiazolines, and thiazolidines. *N*-Methylthiazolidine was identified by NMR spectra in addition to the GC–MS method. Most compounds identified in these model systems have previously been found in foods. Thiazolidines, which are reduced products of thiazoles and thiazolines, have not, however, been found in foods. The formation pathways of these heterocyclic compounds are also discussed.

Thiazole compounds are important flavor constituents in many foods, such as beef, beer, chocolate, coffee, and popcorn (Liebich et al., 1972; Buttery et al., 1967; Stoll et al., 1967; Walradt et al., 1970). A review of thiazoles in foods has been published (Maga, 1975). This review covers almost the entire range of roles of thiazoles in foods. The formation pathways of thiazoles are, however, not yet well understood. Several investigators have postulated reasonable formation pathways for thiazoles. For example, Mussinan et al. (1975) suggested that 2,4,5-trimethyl-3-thiazoline is formed from the reaction of diacetyl, hydrogen sulfide, and ammonia through the intermediate 2-keto-3-butanethiol. Assuming thiazoles form from thiazolines by dehydrogenation, the postulation of Mussinan et al. is quite a reasonable one. Shibamoto and Russell (1976) reported the formation of many thiazoles from the reaction of D-glucose, hydrogen sulfide, and ammonia. They proposed that D-glucose decomposes into various carbonyls, which subsequently react with hydrogen sulfide and ammonia to give thiazoles. The question remains, however: What are the sources of carbon, nitrogen, and sulfur atoms for this unique heterocyclic ring? It is obvious that the source of carbon atoms for thiazoles is D-glucose in the reaction of a glucose–hydrogen sulfide–ammonia model system. On the other hand, some researchers obtained thiazoles from the reaction of cysteine or cystine and ribose (Mulders, 1973; Fujimaki et al., 1969), and they have postulated that carbons 4 and 5 of the thiazole ring come from the amino acid and that carbon 2 comes from the sugar. For example, 2-acetyl-2-thiazoline, which has also been found in beef broth (Tonsbeek et al., 1971), would form from the reaction of cysteamine (decarboxylation product of cysteine) and pyruvaldehyde (product of sugar caramelization). In order to clarify the source of these atoms (C, N, S) of the five-membered heterocyclic ring in food systems, we investigated the reaction products of

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